

Response of soil organic matter dynamics to conversion from tropical forest to grassland as determined by long-term incubation

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Abstract Understanding soil organic carbon (SOC) responses to land-use changes requires knowledge of the sizes and mean residence times (MRT) of specific identifiable SOC pools over a range of decomposability. We examined pool sizes and kinetics of active and slow pool carbon (C) for tropical forest and grassland ecosystems on Barro Colorado Island, Panama, using long-term incubations (180 days) of soil and stable C isotopes. Chemical fractionation (acid hydrolysis) was applied to assess the magnitude of non-hydrolysable pool C (NHC). Incubation revealed that both grassland and forest soil contained a small proportion of active pool C (<1%), with MRT of ~6 days. Forest and grassland soil apparently did not differ considerably with respect to their labile pool substrate quality. The MRT of slow pool C in the upper soil layer (0–10 cm) did not differ between forest and grassland, and was approximately 15 years. In contrast, changes in vegetation cover resulted in significantly shorter MRT of slow pool C under grassland (29 years) as compared to forest (53 years) in the subsoil (30–40 cm). The faster slow pool turnover rate is probably associated with a loss of 30% total C in grassland subsoil compared to the forest. The NHC expressed as a percentage of total C varied between 54% and 64% in the surface soil and decreased with depth to ~30%. Grassland NHC had

considerably longer MRTs (120 to 320 years) as compared to slow pool C. However, the functional significance of the NHC pool is not clear, indicating that this approach must be applied cautiously.

Keywords Active pool carbon · Forest conversion · Grassland · Incubation · Mean residence time · Non-hydrolysable carbon · Slow pool carbon · Stable C isotopes · Panama

Introduction

Soil organic carbon (SOC) is a complex mixture of carbon (C) compounds derived from plants, animals, and microbes, which vary greatly in their amount and decomposition dynamics. However, predictive understanding of SOC responses to climate, and land-use changes requires knowledge of the sizes and mean residence times (MRT) of specific identifiable SOC pools. In models, SOC is often grouped into three conceptual pools to characterize long- and short-term changes in soil C storage and decomposition rates (Parton et al. 1987). The ‘active,’ ‘labile,’ or ‘fast’ pool is assumed to be composed of microbial biomass and easily decomposable compounds (e.g., proteins and polysaccharides) from leaf litter and root-derived material with short MRT (from days to years). The ‘slow’ or ‘intermediate’ pool is understood to consist of refractory components of litter, weakly sorbed carbon, and MRT from 10 to more than 100 years. The ‘passive,’ ‘resistant,’ ‘recalcitrant,’ or ‘stable’ pool is considered to be composed of aliphatic compounds, often mineral-stabilized, with MRT on the order of 10^3 years (Trumbore 1997; Paul et al. 2001; von Lützow et al. 2006).

Different fractionation techniques (biological, chemical, and physical) are employed to isolate SOC pools and to

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assess C dynamics. Long-term laboratory incubation (biological fractionation scheme) is used to approximate the natural process of microbial degradation in the soil to estimate active and slow pool C sizes and their MRTs (Townsend et al. 1997; Paul et al. 2001). This method assumes that microbes will mineralize the most labile C first (Fig. 1, schematic drawing). The slow pool will be mineralized more slowly over a longer period (Townsend et al. 1997; Paul et al. 2001). Active and slow pools are considered to be sensitive to land management (McLauchlan and Hobbie 2004) and depth (Fierer et al. 2003).

Several chemical fractionation methods are used to isolate fractions that represent the passive C pool in soils, the most common of which is acid hydrolysis by 6-M HCl (Leavitt et al. 1996; Six et al. 2002; Plante et al. 2006a). The acid solution hydrolyses compounds that are potentially biodegradable, e.g., proteins, nucleic acids, or polysaccharides (=hydrolysable fraction), while more recalcitrant macromolecules (e.g., highly aliphatic compounds) remain in the residue (=non-hydrolysable C, NHC). However, the resistance of compounds to laboratory hydrolysis may not be paralleled by a strong resistance to natural biological degradation in the soil (Poirier et al. 2006; Helfrich et al. 2007), suggesting that hydrolysis may not accurately quantify resistant soil C (Balesdent 1996; Trumbore and Zheng 1996).

Combining fractionation techniques with ^{13}C natural abundance offers a tool to test the contribution of different soil organic matter fractions to mineralization (Paul et al. 2001). The ^{13}C natural abundance technique can be applied in plant/soil systems where the photosynthetic pathway of the vegetation has changed (Balesdent and Mariotti 1996), for example, where C_4 grass (average $\delta^{13}\text{C}$ value of -11‰) grows on soils derived from C_3 vegetation (average $\delta^{13}\text{C}$ value of -27‰ ; Smith and Epstein 1971).

In Central Panama as well as other parts of the humid tropics, the native tropical forest is undergoing a rapid conversion into agricultural land, mainly pastures (Condit et al. 2001). The present study provides a unique opportunity to assess how changes in vegetation can affect soil C stocks and dynamics, independent of land management such as cropping or grazing. Previous measurements on water stable aggregates and density fractions at the same study sites revealed that simply altering the vegetation cover did not alter the soil aggregate structure but led to a loss of about 25% of total SOC (0–50 cm depth; Schwendenmann and Pendall 2006). In the present study, we conducted long-term incubations to quantify changes in pool sizes and MRT of active and slow C pools in forest and grassland soils taken from different depths. Furthermore, we estimated the non-hydrolysable C pool to assess if this approach provides meaningful resistant pool C and allows insight into carbon stabilization at the sites in question. We also measured stable C isotopes in the different pools to quantify changes of C resulting from vegetation

change. We expected that active pool C and its MRT would differ between forest and grassland soils but that slow pool C would be less affected and that these changes would be soil-depth-dependent. Results from this study should improve models predicting effects of tropical forest conversion to grasslands on C cycling at local to global scales.

Material and methods

Site description

The study was conducted on Barro Colorado Island (BCI; $9^{\circ}10' \text{ N}$, $79^{\circ}51' \text{ W}$), which is located in the middle of the Panama Canal. The mean annual temperature of the region is 26°C (STRI 2006). Average total yearly rainfall is 2,600 mm per year, with a distinct dry season (approximately from mid-December to the end of April). The vegetation on BCI is classified as Tropical Moist Forest in the Holdridge life zone system (Leigh et al. 1996). Our study sites were located on the north side of the island close to the Miller lighthouse (UTM-626284 E, 1013458 N). The soils are oxisols, developed in andesite parent material (Yavitt 2000), with clay mineralogy dominated by kaolinite (Schwendenmann and Pendall 2006). We selected two different land-use types, undisturbed forest and grassland. The forest site appeared undisturbed by pre-twentieth century clearing (Condit et al. 1996). At the adjacent grassland site, the forest was cut around 90 years ago to set up a lighthouse for the Panama Canal and has been maintained by burning and cutting. At the time of sampling, the vegetation of the grassland site was dominated by wild sugarcane (*Saccharum spontaneum*), a C_4 plant. Soil texture in both land-use types was sandy clay loam throughout the profile. Small macroaggregates (250–2,000 μm) accounted for 60% to 80% of the total sample mass under forest and grassland (Schwendenmann and Pendall 2006). In the upper layers (0- to 5- and 5- to 10-cm depth), pH ranged from 4.9 (forest) to 6.6 (grassland), and in the subsoil (30- to 40-cm depth), pH was 4.9 for forest and 4.6 for grassland. The higher pH in surface grassland soil is most likely due to regular burning. The soil profile (to 50-cm depth) contained between 64 ± 8 (grassland) to 84 ± 9 (forest) Mg C ha^{-1} ($P > 0.05$; Schwendenmann and Pendall 2006).

Field sampling and soil analyses

We selected three paired plots at upper, middle, and toe-slope positions in forest and grassland. The plots were separated by about 200 m along the slope and by ~ 100 m between forest and grassland. Samples were taken from one soil pit per plot from the dark reddish brown A horizon (0- to 5- and 5- to 10-cm depth) and the reddish, stoney B horizon (30- to 40-cm depth) in January 2004 (beginning of dry season). For the

incubation experiment, field-moist subsamples were kept at 4°C until analysis. To estimate the soil field moisture content, subsamples were dried at 105°C for 48 h.

Incubation experiment

Incubations were performed using 15 (0- to 5-cm depth) or 20 g (5- to 10- and 30- to 40-cm depth) field-moist soil. Before incubation, the soil was sieved (8 mm), and roots were removed. Duplicate sub-samples from each of the three replicate profiles were placed in 50-ml polystyrene beakers, which had small holes punched into the bottom, and pre-combusted glass-fiber filter paper lining the beaker for drainage. The soil was gently compressed to simulate field bulk density, which ranged between 0.85 Mg m⁻³ and 0.99 Mg m⁻³, and did not differ significantly among depths or between land-use types (Schwendenmann and Pendall 2006). Soil-filled and blank jars were flushed with dry, compressed air (CO₂ concentration, 377 ppm; $\delta^{13}\text{CO}_2 = -44\text{‰}$) and then incubated in dark growth chambers at 25°C. The CO₂ production was periodically (at intervals ranging from 1 to 20 days) determined using an infrared gas analyzer (LI-820, LI-COR, Lincoln, Nebraska, USA) on 15-ml headspace samples. A calibration curve (power function) was established with standards of 372, 1,000, and 2,700 ppm CO₂. Samples ranged between 500 and 5,000 ppm CO₂. After each sampling, the jars were flushed with compressed air. When necessary, deionized (DI) water was added to maintain the original field moisture content, which ranged between 30% and 50%. For similar soil types, soil respiration rates were highest at soil moisture contents between 30% and 50%, indicating the optimal range for microbial activity (Schwendenmann et al. 2003).

Carbon mineralization rates were expressed on a per unit oven-dry soil basis (microgram C per gram soil per day) and on a per unit total C (C_t) basis (milligram C per gram C_t per day). The cumulative CO₂ mineralized (C_{cum}) was estimated by linear interpolation between sampling dates and adding up the daily mineralization rates.

Acid hydrolysis

The resistant carbon fraction was determined by acid hydrolysis (Paul et al. 2001), using a flat bottom flask with 2 g soil (air-dry and root-free, <8 mm) and 50 ml 6-M HCl. The soil/acid mixture was boiled at 100°C for 18 h using a heating plate. After boiling, the suspension was filtered, and the remaining solid fraction on the filter paper was rinsed with DI water. The residue was dried, weighed, and ground for C and ¹³C analyses.

Previous studies indicate that major plant components like lignin and cellulose (Kögel-Knabner et al. 1994) are resistant to acid hydrolysis. Thus, in a subset of samples, the light

fraction, which is considered to be mainly composed of fresh organic residues, was removed by density fractionation using sodium polytungstate (SPT) with a density of 1.85 g cm⁻³. After density fractionation (pretreatment), samples were washed with distilled water and then subjected to acid hydrolysis as described above.

Isotopic composition of carbon pools

The C content and ¹³C were measured for pre- and post-incubated soil samples and non-hydrolysable material using high-temperature combustion (NCS 2500, CE Elantech, Lakewood, NJ, USA) and an isotope-ratio mass spectrometer (Isoprime, Micromass, Manchester, UK). Stable C carbon isotope ratios are expressed as:

$$\delta^{13}\text{C} (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ and the standard was V-PDB. The precision of the $\delta^{13}\text{C}$ analysis was $\pm 0.2\text{‰}$.

Variations in $\delta^{13}\text{C}$ of the CO₂ that evolved from the surface soil layer (0–5 cm) during the incubations were determined using gas chromatography–isotope ratio mass spectrometry (GC-IRMS, Micromass, Manchester, UK). Respired ¹³CO₂ signature was measured periodically between 1 and 110 days after the start of the incubation as described by Pendall and King (2007).

Active and slow pool sizes and mean residence times

Daily mineralization rates were used to calculate active and slow pool sizes and turnover rates based on the two-pool constrained model described by Paul et al. (2001; Eq. 2).

$$\frac{dC}{dt} = C_a e^{-(k_a \times \text{days})} + C_s e^{-(k_s \times \text{days})} \quad (2)$$

where dC/dt was the daily C mineralization rate, C_a is the size of the active pool, k_a is the turnover rate for the C_a pool, C_s is the size of the slow pool, k_s is the turnover rate for the slow pool. The slow pool comprised the total C pool minus the sum of active pool C and NHC (pretreated samples). Active pool size and turnover rates (k) per day of active and slow pool C were estimated by applying the exponential decay model (Eq. 2), using a nonlinear regression (PROC NLIN, Method = Marquardt, SAS, version 8.2, SAS Institute Inc. Cary, NC, USA) that adjusted for the curvilinear relationship of the C mineralization between sampling points (Paul et al. 2001). C_a and C_s were expressed on a per unit total C basis ($\mu\text{g C g}^{-1} C_t$). We report the turnover rate as MRT (in days or years), which is an inverse function of the turnover rate (k^{-1}).

Proportion of grassland-derived C of respired CO₂ and non-hydrolysable carbon

The $\delta^{13}\text{C}$ values of respired CO₂ from active pool C were used to estimate the proportion of “new” C [grassland-derived C (C_4), incorporated to the soil since vegetation change] utilized by microbes. We applied a simple mass-balance approach (Balesdent and Mariotti 1996), assuming that the ‘new’ C (C_{new}) was isotopically similar to fresh grass residue and that ‘old’ C was similar to $\delta^{13}\text{C}$ of carbon in pre-incubated forest soil.

$$\%C_{\text{new}} = \frac{(\text{active pool } \delta^{13}\text{CO}_{2\text{grassland}} - \delta^{13}\text{C}_{\text{pre-incubated forest soil}})}{(\delta^{13}\text{C}_{\text{grass residue}} - \delta^{13}\text{C}_{\text{pre-incubated forest soil}})} \times 100 \quad (3)$$

We considered the $\delta^{13}\text{C}$ of C_a under grassland to be characterized by CO₂ that evolved over the first 6 days of the incubation period (based on the MRT of C_a), although we corrected this for the contribution of slow pool CO₂ as described by Pendall and King (2007).

The proportion of C_4 (% NHC_{new}) for grassland NHC was calculated using Eq. 3, where forest NHC was used as ‘old’ C and C_4 residue ($-16.2\text{‰} \pm 4.2\text{‰}$) as ‘new’ C. The MRT (in years) for NHC was estimated using a first-order decay model (Six and Jastrow 2002).

$$\text{MRT}_{\text{NHC}}(\text{years}) = \frac{-t}{\ln\left(\frac{C_t}{C_0}\right)} \quad (4)$$

where t is the time since conversion (here 90 years), C_t is the content (in g C kg⁻¹) of the remaining forest-derived NHC in the grassland soil at the time of sampling, and C_0 is the NHC content present under the forest at the time of sampling.

Statistical analyses

Differences in C mineralization, pool sizes, MRT, and $\delta^{13}\text{C}$ among depths and between vegetation types were tested employing a two-way analysis of variance and Tukey’s honestly significant differences post hoc test ($n=3$, $P<0.05$). We used Pearson product-moment correlation to identify relationships among parameters. Statistical analyses were conducted with Statistica 7.1 (StatSoft, Tulsa, OK, USA).

Results

Carbon mineralization

The emission of carbon dioxide (per gram soil C) from incubated forest and grassland soils followed the same general pattern (Fig. 1), with highest rates from surface soils and lowest rates from subsoils. Respiration rates were about 0.6 to 1.4 mg C per gram C per day during the initial active pool phase, which lasted approximately 10 days. The rates dropped considerably after 4 weeks and remained relatively constant at less than 0.2 mg C per gram C per day during the slow pool phase (Fig. 1). Highest rates were observed in surface soils, indicating higher microbial activity and lowest rates in forest subsoils.

Cumulative C mineralization in 0- to 5-cm depth was 1,151 μg C per g soil per 180 days in the forest and 1,130 μg C per gram soil per 180 days in the grassland soil ($P>0.05$; Table 1) or 28 mg C per gram C per 180 days for both forest and grassland soil. This represented a loss between 2.6% (forest) and 2.9% (grassland) of total soil C during the 6 months of incubation. The percentage of total C mineralized in subsoil samples was significantly higher in grassland than forest (1.5% vs. 0.9%, respectively; Table 1). Total cumulative C mineralization was closely correlated with total soil C content (grassland, $r^2=0.90$; forest, $r^2=0.99$).

Active and slow pool sizes and mean residence times

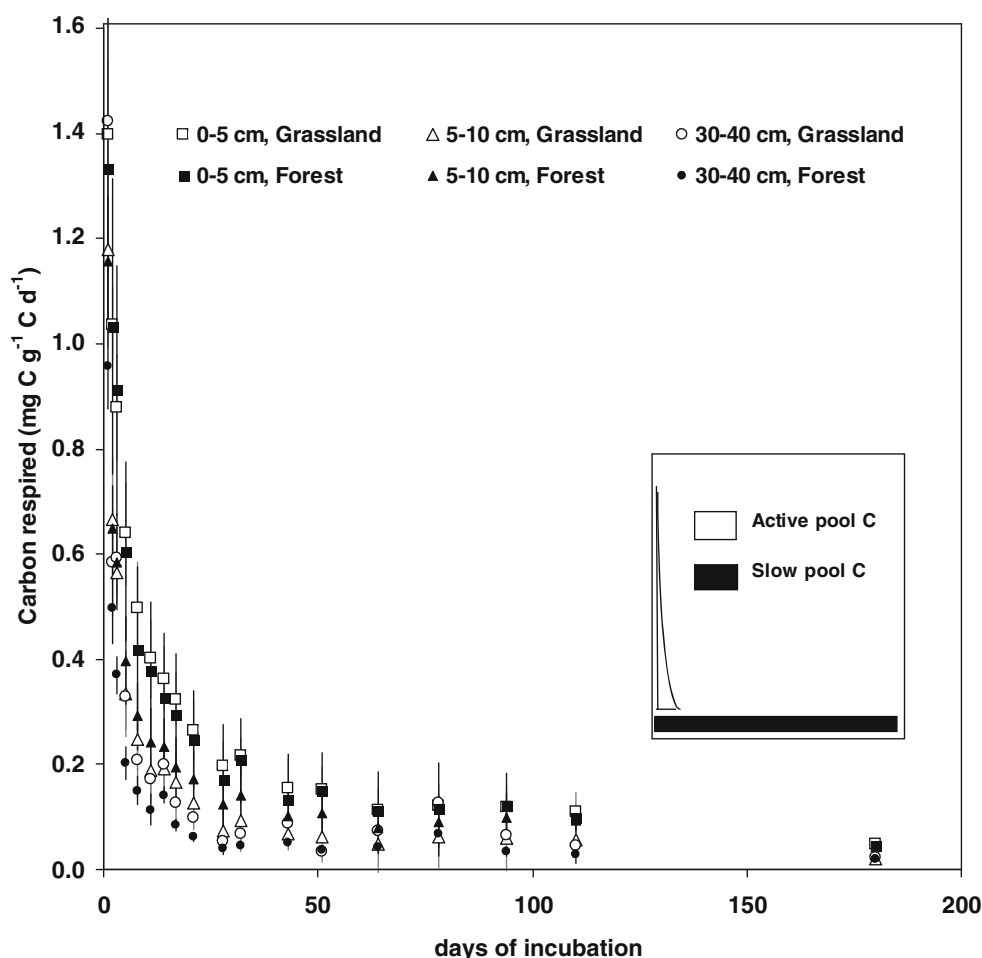
Based on the curve analysis (Eq. 2), active pool C was <1% of soil C and did not differ between grassland and forest soils (Table 2). The highest proportion of active pool C (0.7% under forest and 0.9% under grassland) was found in 0- to 5-cm depth; significantly less was found in the subsoil, with 0.4% in grassland and 0.3% in forest.

The MRT of active pool C in the surface soil (0–5 cm) was approximately 6 days for both forest and grassland, and decreased significantly with depth (Table 2). The MRT of slow pool C was 12 years in surface soil in the grassland and 15 years in the forest ($P>0.05$). Under both vegetation types, slow pool MRT increased with depth. A considerably longer MRT was estimated for the forest subsoil (53 years) as compared to the grassland soil (29 years; $P<0.05$).

Non-hydrolysable carbon

The NHC (no pretreatment) represented 54–64% of the total C in the surface soil under both land-use types (Table 3). The proportion of non-hydrolysable C decreased significantly with depth. In the subsoil, NHC made up a

Fig. 1 C mineralization rates over the course 180 days for forest and grassland soil (0- to 5-, 5- to 10-, and 30- to 40-cm), Barro Colorado Island, Panama. Error bars are ± 1 standard deviation, $n=3$. The daily mineralization rates were used as inputs for the two-pool model (Eq. 2)



significantly higher proportion of grassland SOC (32%) than of forest SOC (27%; Table 3).

Removing low-density organic debris by density fractionation before hydrolysis tended to result in a lower proportion of NHC in 0- to 5- and 5- to 10-cm depth under forest. Under grassland (0–5 cm) pretreatment of samples led to a significant decrease of NHC (Table 3).

Isotopic composition

The total C (no pretreatment) $\delta^{13}\text{C}$ signature differed significantly between forest (-25.0 to -28.0‰) and grassland (-21.4 to -23.5‰ ; Table 4) because the forest was dominated by C_3 trees (forest litter= $-30.1\pm 0.5\text{‰}$), and the grassland was dominated by C_4 grasses (grass residue= $-16.2\pm 4.2\text{‰}$).

Table 1 Total C content and cumulative amount of mineralized C from forest and grassland soil after 180 days of incubation, Barro Colorado Island, Panama (values in parentheses are ± 1 standard deviation, $n=3$)

Site	Depth (cm)	Total C (g C kg ⁻¹)	Mineralized C		
			Soil basis ($\mu\text{g C g}^{-1}$ soil per 180 days)	C basis (mg C g ⁻¹ C per 180 days)	Percent of total C (%)
Forest	0–5	44.7 a A (14.1)	1151 a A (479)	28.5 a A (14.4)	2.6 a A (0.8)
	5–10	23.5 b A (4.7)	480 a A (196)	21.3 a A (8.5)	2.0 a A (1.0)
	30–40	8.9 c A (1.6)	84 b A (20)	10.2 b A (2.4)	0.9 b A (0.4)
Grassland	0–5	38.2 a A (6.9)	1130 a A (299)	28.1 a A (7.1)	2.9 a A (1.1)
	5–10	27.3 b A (1.7)	424 a A (275)	15.2 a A (8.8)	1.6 ab A (0.8)
	30–40	5.2 c B (0.9)	80 b A (20)	16.0 b A (4.1)	1.5 b B (0.2)

Different small letters indicate differences among depths for each land-cover type; different capital letters indicate differences between land-cover types for that depth ($P<0.05$).

Table 2 Pool sizes and mean residence times (MRT) determined from long-term incubation of forest and grassland soil from 0- to 5-, 5- to 10-, and 30- to 40-cm depths, Barro Colorado Island, Panama (values in parentheses are ± 1 standard deviation, $n=3$)

Site	Depth (cm)	Total C (g C kg ⁻¹)	Active C		Slow C ^a	
			% of total C	MRT days	% of total C	MRT years
Forest	0–5	44.7 a A (14.1)	0.7 a A (0.2)	5.9 a A (1.3)	36.2 a A (3.3)	14.7 a A (3.1)
	5–10	23.5 b A (4.7)	0.4 b A (0.1)	3.2 b A (1.0)	42.7 a A (3.0)	16.3 a A (5.4)
	30–40	8.9 c A (1.6)	0.3 c A (0.1)	2.0 b A (0.5)	72.4 b A (5.8)	53.2 b A (6.1)
Grassland	0–5	38.2 a A (6.9)	0.9 a A (0.1)	6.4 a A (1.8)	35.1 a A (4.7)	11.7 a A (2.2)
	5–10	27.3 b A (1.7)	0.4 b A (0.1)	2.9 b A (0.9)	46.0 a A (3.0)	22.3 b A (8.3)
	30–40	5.2 c B (0.9)	0.4 b B (0.2)	1.7 b A (0.1)	67.1 b A (6.1)	29.4 b B (4.1)

Different small letters indicate differences among depths for each land-use type; different capital letters indicate differences between land-use types for that depth ($P<0.05$).

^a Slow C = total C – active pool C – non-hydrolysable C (pretreated)

The $\delta^{13}\text{C}$ values of forest and grassland soil did not change significantly during the incubation (Table 4). All samples that were subjected to density fractionation (pretreatment) were slightly more enriched in ^{13}C as compared to samples without removal of organic debris (Table 4). Pretreatment slightly reduced the proportion of new C and increased the MRT in subsoil (Table 3).

Independent of pretreatment and vegetation type, the $\delta^{13}\text{C}$ value of NHC residue was more negative than total C (Table 4). The $\delta^{13}\text{C}$ values of total C and NHC became more positive with depth in the forest but more negative in the grassland (Table 4). The proportion of C₄ derived NHC under grassland was 46% in 0–5 cm and 17% in 30–40 cm (Table 3). The MRT of NHC ranged between 120 years (0–5 cm) and 318 years (30–40 cm; Table 3).

The $\delta^{13}\text{C}$ signatures of respired CO₂ reflect the C substrates utilized during active and slow pool phases of the incubation (Fig. 2). The $\delta^{13}\text{C}$ respired from active pool C (corrected for the contribution of slow pool $\delta^{13}\text{C}$) was

$-18.2\pm 1.4\text{‰}$ under grassland and $-24.2\pm 3.9\text{‰}$ under forest. Slow pool C ($-27.2\pm 1.1\text{‰}$) under forest was slightly more negative than active pool C ($P>0.05$), whereas under grassland, slow pool C ($-16.2\pm 2.4\text{‰}$) was similar to active pool C ($P>0.05$). In the grassland, the CO₂ respired from the active- and slow-pool C was derived primarily (>80%) from the actual C₄ vegetation (Eq. 3).

Discussion

Pools and kinetics of carbon decomposition

Under both land-cover types, the proportion of active pool C was small (<1%) and MRTs were short (~10 days), leading to rejection of our first hypothesis that active pool size and its MRT would be most affected by the conversion from forest to grassland. Forest and grassland soil apparently did not differ considerably with respect to their labile

Table 3 Fraction of non-hydrolysable carbon (NHC) of forest and grassland soil, proportion of new NHC, and mean residence time (MRT) of NHC in grassland soil (no pretreatment vs. pretreatment to

remove light fraction organic matter) of from 0- to 5-, 5- to 10- and 30- to 40-cm depths, Barro Colorado Island, Panama (values in parentheses are ± 1 standard deviation)

Site	Depth (cm)	No pretreatment				Pretreatment			
		NHC		NHC _{new}	MRT	NHC		NHC _{new}	MRT
		g C kg ⁻¹	% of total C			g C kg ⁻¹	% of total C		
Forest	0–5	27.8 a A (5.2)	63.1 a A ¹ (3.8)			21.0 A (2.3)	57.8 ¹ (6.6)		
	5–10	16.7 a A (3.3)	56.9 a A (5.2)			10.5 (–)	39.8 (–)		
	30–40	2.8 b A (1.0)	27.3 b A (1.7)			2.5 (–)	27.0 (–)		
Grassland	0–5	24.5 a A (4.0)	64.0 a A ¹ (5.2)	46.4	119.6	19.2 A (1.1)	54.0 ² (2.6)	46.3	122.5
	5–10	15.8 a A (2.3)	53.6 b A (3.5)	32.1	260.3	17.3 (–)	53.8 (–)	37.3	No data
	30–40	2.6 b A (0.9)	32.5 c B (0.7)	17.0	317.7	2.3 (–)	32.4 (–)	12.0	508.9

Different small letters indicate differences among depths for each land-use type; different capital letters indicate differences between land-use types for that depth; significant differences between NHC (no pretreatment vs. pretreatment) are denoted by different numbers ($P<0.05$).

Table 4 $\delta^{13}\text{C}$ values of total C and non-hydrolysable carbon (NHC) of forest and grassland soil from 0- to 5-, 5- to 10-, and 30- to 40-cm depths, Barro Colorado Island, Panama (values in parentheses are ± 1 standard deviation, $n=3$)

Site	Depth (cm)	Total C			NHC	
		No pretreatment	Pretreatment	After incubation, $\delta^{13}\text{C}$ (‰)	No pretreatment	Pretreatment
Forest	0–5	–28.0 a A ¹ (0.5)	–25.9 A ² (0.9)	–27.2 a A ^{1,2} (0.3)	–30.0 a A ³ (0.4)	–29.6 A ³ (0.7)
	5–10	–26.9 a b A ¹ (0.6)	–25.0 (–)	–26.1 a A ¹ (0.4)	–29.1 ab A ¹ (1.4)	–29.4 (–)
	30–40	–25.0 b A ¹ (0.1)	–23.0 (–)	–24.8 b A ¹ (0.7)	–26.0 b A ² (0.2)	No data
Grassland	0–5	–21.4 a B ¹ (0.9)	–20.3 B ¹ (0.3)	–21.9 a B ¹ (0.9)	–23.5 a B ¹ (1.1)	–23.3 a B ¹ (0.1)
	5–10	–22.4 a B ¹ (0.8)	–21.6 (–)	–22.7 a B ¹ (1.2)	–24.9 a B ² (0.7)	–24.4 (–)
	30–40	–23.5 b B ¹ (0.6)	–23.0 (–)	–22.4 a A ¹ (1.4)	–24.3 a B ² (0.1)	–24.8 (–)

Different small letters indicate differences among depths for each land-use type; different capital letters indicate differences between land-use types for that depth; significant differences between values in rows are denoted by different numbers ($P<0.05$).

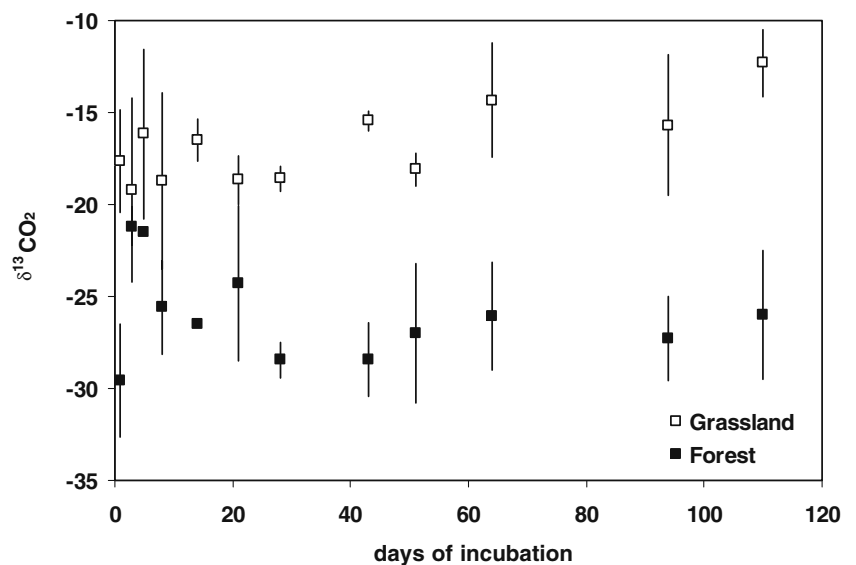
pool substrate quality. Active pool C may have been comprised mainly of metabolic cell contents that did not differ in quality between the vegetation types.

Our results on the proportion of active pool C ($<1\%$, Table 2) were on the low end of most reported values (0.5–8%) from long-term incubation studies on temperate and tropical forest and grassland soils (Townsend et al. 1997; Haile-Mariam et al. 2000; Torn et al. 2005). At a 50-year-old pasture site approximately 40 km east of the study area, active pool carbon was 1.2% of C_t (Schwendenmann et al. 2007). Differences in active pool C sizes among sites may be attributable to soil clay content. Gregorich et al. (1991), Ladd et al. (1996), and Franzluebbers (1999) observed a positive correlation between clay content and microbial biomass. Although the clay content of our soils was considerable ($\sim 30\%$), active pool size was low. This is in agreement with Torn et al. (2005) who did not find a correlation between soil texture, carbon pool sizes, and turnover times in Hawaiian soils of different soil mineralogy.

Active pool C MRTs of the studied forest and grassland surface soils were shorter than values reported in other studies (Paul et al. 2006). The data compiled by Paul et al. (2006) are from temperate grassland soils. Šantrůčková et al. (2000) compared microbial processes in tropical and temperate grassland soils, and reported that the turnover rate of organic material may be faster in tropical soils as compared to temperate soils. The MRTs between 2 and 6 days found in our experiment can be compared with MRTs of glucose (1 day) and fungal cytoplasm (10 days; Paul and Clark 1996).

The MRT of active pool C decreased significantly with depth under grassland and forest. The MRT of active C under grassland tended to be lower, whereas the percentage of total C mineralized in subsoil was significantly higher under grassland (1.5%) than forest (0.9%). This reinforces that C in grassland at depth is more labile than in forest. A larger contribution of roots and root exudates may explain the more labile nature of C at depth in grassland soil. It is unlikely that sampling disturbance would explain shorter MRTs in deeper

Fig. 2 The $\delta^{13}\text{C}$ signature of CO_2 respired for forest and grassland soil (0–5 cm), Barro Colorado Island, Panama. Error bars are ± 1 standard deviation, $n=3$



layers (Ewing et al. 2007) as the soil under forest and grassland mainly consisted (60% to 80%) of small macro-aggregates (250–2,000 μm ; Schwendenmann and Pendall 2006), which were not disturbed by using a large sieve.

The MRTs of slow pool C in the top 10 cm of the forest were similar to slow pool C MRTs estimated in other moist tropical forests. Soil incubations revealed that the slow pool of Andisols in Hawaiian pastures and forests had MRTs of 4–20 years (Townsend et al. 1995; Torn et al. 2005). Near Manaus, Brazil, the slow pool C had MRTs of 5–10 years (Telles et al. 2003), as estimated through radiocarbon data. Our hypothesis that slow pool C would be less affected by vegetation change was supported for upper soil layers, as slow pool MRT did not differ significantly between forest and grassland.

However, the slow pool MRT was significantly shorter in grassland (29 years) than forest subsoil (53 years), possibly because substrate quality and root exudation were higher in grassland at that depth. Fontaine et al. (2007) suggest that priming by the input of fresh-plant-derived carbon to the subsoil can increase decomposition of deeper soil C. Faster slow pool turnover of grassland SOC in deeper layers is consistent with significantly lower total SOC concentration in grassland subsoil (Table 1), especially considering that 70% of the total pool is slow C in subsoil.

Non-hydrolysable carbon

There is increasing concern about the suitability of HCl hydrolysis to isolate older C fractions because fresh plant material can resist hydrolysis, whereas older C components, possibly stabilized by mineral association, may be selectively hydrolyzed (Balsedent 1996; Helfrich et al. 2007). The NHC expressed as a percentage of total C varied between 54% and 64% in the surface soil (0–5 cm) and decreased with depth to ~30% at 30–40 cm. Removing organic debris by density fractionation before hydrolysis tended to slightly reduce the fraction of NHC. In pretreated samples, Plante et al. (2006b) found in 0–5 cm depth a NHC proportion of 32–50% under native grassland in Saskatchewan and 41–55% from soil developed under forest in Ohio. A recent review based on the analysis of over 300 data points (forest, grassland, and cultivated soils; 0- to 10-cm depths) showed that the C remaining after hydrolysis with 6-M HCl (at 116°C) ranged from 30% to 80% of the total C (Paul et al. 2006).

In both forest and grassland soils, NHC decreased with depth. This was also reported by Paul et al. (2006) for cultivated conventional tillage and forested sites from different climatic regions. We found that the proportion of NHC was strongly correlated to the amount of total C (grassland, $r^2=0.91$; forest, $r^2=0.84$). Plante et al. (2006a) also observed a strong correlation of C concentrations in the NHC fraction

with total C content. This relationship might partly explain the differences in NHC between surface soil and subsoil.

Forest NHC had more negative $\delta^{13}\text{C}$ values than total C, suggesting that the 6-M HCl digest method concentrates lignin and/or lipids (alkyl-C) relative to the bulk soil (Preston et al. 1997; Plante et al. 2006a) because both these constituents are depleted in ^{13}C relative to whole plant tissue (Benner et al. 1987). The isotopic signature of NHC under forest ($-29.6\pm0.70\text{‰}$ to $-30.0\pm0.38\text{‰}$) was similar to free light fraction ($<1.85\text{ g cm}^{-3}$; -29.2 to -31.3‰ ; Schwendenmann and Pendall 2006), also supporting the idea that NHC contains recently deposited plant material. Under grassland, alkyl-C left after biomass burning may contribute a sizable fraction of grassland NHC due to its recalcitrant nature (Lorenz et al. 2007). Alternatively, the more negative $\delta^{13}\text{C}$ signal of grassland NHC as compared to total C could indicate that grassland NHC still contains a considerable amount of forest-derived C, which agrees with the longer MRT of grassland NHC. Our data indicate that MRT of grassland NHC (120 to 320 years, no pretreatment) is considerably longer than slow pool C (12 to 29 years by the incubation method) and bulk soil C (70 to 190 years by the ^{13}C method; Schwendenmann and Pendall 2006). The MRT data and the relatively small change observed with pretreatment support the idea that the hydrolysis residue is distinctly older than the slow pool and largely derived from forest C.

In the upper 10 cm, we did not observe a significant difference between forest and grassland with respect to the proportion of NHC. In contrast, the proportion of NHC at 30–40 cm depth was significantly higher under grassland as compared to forest (Table 3). This observation has to be treated with care as our data set is small. Nonetheless, our results are in agreement with Parton et al. (1987) who suggest that resistant pool C should be a greater proportion of the total C under pasture or cultivated land as a result of a loss of active and slow pool C due to enhanced mineralization caused by land-use change. Our results demonstrate that acid hydrolysis after density fractionation can result in an old C pool, but more detailed structural analysis such as with ^{13}C -NMR would provide further insight into the nature of the resistant soil C pool (Nelson and Baldock 2005).

Changes of $\delta^{13}\text{C}$ during decomposition

We expected differences in $\delta^{13}\text{C}$ among carbon pools that would allow us to gain information on decomposition dynamics. Respired CO_2 from forest soil was more enriched in ^{13}C than total C (before incubation) and plant residue (Table 4, Fig. 2). We suggest that this might be caused by selective utilization of ^{13}C -enriched compounds, such as carbohydrates or polysaccharides (Gleixner et al. 1999). Furthermore, the active pool consists mainly of microbial

matter, which is enriched in ^{13}C (Werth and Kuzyakov 2008). It is unlikely that isotopic discrimination accompanying heterotrophic metabolism (Šantrůčková et al. 2000) explained this pattern, in part because the continuous loss of ^{13}C -enriched compounds during incubation did not result in ^{13}C -depletion in soil C (after incubation).

In the grassland, CO_2 respired throughout the incubation was almost entirely C_4 -derived (>80%). As the conversion from forest to grassland took place 90 years ago, the forest-derived mineralizable C in 0- to 5-cm depth was nearly all replaced by grassland C. We expected to observe a decrease in $\delta^{13}\text{C}$ values of respired CO_2 concurrently with the drop in mineralization rates as evidence that some forest C remained in the slow pool. Instead, the microbial respiration in both grassland and forest soils appeared to be close to equilibrium with the current vegetation at each site during both the active and slow pool phases.

Evaluation of methods

Laboratory incubation combined with acid hydrolysis was applied to quantify and characterize active, slow, and passive soil C pools. Non-linear curve fitting analysis after Paul et al. (2001) provided reasonable results for active pool size and the active and slow pool mean residence times. Slow pool C is defined as the difference between total C and the sum of active C and the C found in the acid hydrolysis residue (NHC). Thus, slow pool C and its respective mean residence time are also influenced by the magnitude of the NHC pool. With acid hydrolysis, we were able to isolate an older C fraction, which in the case of the grassland, also contains a larger fraction of the previous forest vegetation. It remains questionable if the 6-M HCl extraction method is appropriate to quantifying resistant pool C in part because pretreatment to remove light fraction material had inconsistent effects on NHC content and its $\delta^{13}\text{C}$ and MRT. Thus, slow pool C and its turnover rates have to be interpreted carefully.

Although laboratory incubation has some shortcomings, it is one of the few methods providing insight into active and slow pool C dynamics. In an earlier study, we used physical fractionation (separation of water stable aggregates and density fractions) coupled with carbon isotope techniques to assess carbon pools and mean residence times of the same soils (Schwendenmann and Pendall 2006). The MRT of grassland bulk soil C was considerably longer (70 years in 0–5 cm and 190 years in 30–40 cm; Schwendenmann and Pendall 2006) as compared to slow pool MRT estimated by incubation-acid hydrolysis method. Intra-aggregate particulate organic matter of grassland soil had MRT (29–50 years for the top 10 cm; Schwendenmann and Pendall 2006) closest to the MRT estimated for slow pool C in the present experiment. Using physical, chemical, and biological fractionation techniques produced obviously distinct C pools as

demonstrated by their different MRTs. However, von Lützw et al. (2007) warn that most methods still yield C pools, which are heterogeneous in terms of turnover times.

These results imply that increased conversion of tropical forests to grassland vegetation can lead to substantial losses of belowground C. Recent evidence that terrestrial C sinks are becoming weaker in recent years (Canadell et al. 2007) could be partly explained by decadal scale responses of C cycling to twentieth-century land-use change.

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